# PHARMACEUTICAL APPLICATIONS OF A PINYON OLEORESIN

by

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# I. INTRODUCTION

The pinyon or nut pine comprises a major portion of the Pygmy forest flora of the south-western section of the United States. Tens of thousands of acres of pinyon pines occur in continuous belts. Indeed, in the Navajo Indian Reservation alone, there has been estimated to exist over three million acres of pinyon-juniper woodland (Clark and Melis, 1953).

The Indians and Mexicans have utilized this readily available tree for centuries. The nuts have been used by them as a dietary staple, particularly during the winter months (Peattie, 1953). Early settlers, trappers, and explorers are known to have sought out the pinyon nut as a necessary supplement to their otherwise meager ration (Fremont, 1845). Pinyon nuts have since been shown to be a good source of protein, carbohydrate and fat (Long, 1941). If left unshelled, these small seeds may be kept for over a year after roasting without becoming rancid (Peattie, 1953).

The Indians have long utilized the caulking properties of pinyon oleoresin. The Apaches made their baskets water-tight with it. The Navajo still do the same with their water

bottles, and also use it to make a black dye for their wool. The wood of the pinyon has been used as a source of fuel. The structural timber used in the famed pit houses of the southwestern Indians was also pinyon. From a study of the growth rings of this wood, it has been possible to date these pre-historic American homes, and place their building as long ago as 400 - 900 A. D. (Peattie, 1953).

The importance of the pinyon pine to the Indian during the period of intrusion by the first whites was pointed out by John Muir (1912). He states, "This. . . is the Indians' own tree, and many a white man have they killed for cutting it down."

There have been several studies devoted to the development of possible commercial uses of western pinyon pines. The latest of these was concerned with determining whether sufficient volatile oil could be obtained from Utah pinyon pines to be of commercial value as an agent for perfuming pharmaceutical and cosmetic preparations, and to determine the physical and organoleptic properties of such oils (Cole, 1956). Just previous to this work, and as a part of the "surveys and resources studies", The Bureau of Indian Affairs commissioned Deaver and

Haskell, through the University of Arizona, to conduct a study on the pinyon resources on the Navajo and Hopi Indian reservations of Arizona and Utah. This work was confined to possible economic exploitation of the oleoresin, resin, and turpentine of Pinus edulis Engelm. as a competitor of the south-eastern pine for the naval stores market (Deaver and Haskell, 1955). The possibility of using western pines as a source of naval stores products was investigated by the United States Department of Agriculture Forest Service (Betts, 1912; Schorger 1915). Pinus ponderosa, var. scopulorum Engelm., Pinus ponderosa Laws. and Pinus edulis were studied. Using the hack and cup method of collection, Pinus edulis was found to yield an average of 0.147 pounds per cup per week, with the best yield occurring during the months of August and September. The turpentine content averaging 20.6% of the total oleoresin collected. The turpentine was found to contain mostly pinene, and also. . . "anoth oil of quite different properties" which were not described. Schorger (1913) described the oleoresin as having an odor charac teristic of Thuja leaf oil.

Cannon (1953) reported on a correlation shown to exist between uranium content in the needles and peeled twigs of piny

pines and known ore deposits, thus pinyon has been used as a means of locating uranium ore.

The oleoresin, commonly known as pinyon gum, has been reported as having been used by both Indians and early white settlers for many and varied medicinal purposes. The medical lore, passed down by word of mouth, indicates that the oleoresin has been successfully used locally as an antiseptic, rubifacient, counterirritant, as a drawing salve, and as a poultice for toothaches. The "gum" has been used internally both as a stool-softening laxative and as a treatment for various other gastrointestinal disorders.

In view of the numerous forms in which pinyon oleoresin has been utilized medicinally, it was thought important to incorporate the oleoresin and resin, and the oils of the hybrid described by Cole (1956) into various classes of pharmaceutical and dental products. Pharmaceutically elegant preparations of pinyon products could be prepared for the number of persons currently using the crude materials, and perhaps an even greater market might be developed.

If a sufficient demand could be made for these new preparations as well as demonstrating a superiority of pinyon resin, oil

and oleoresin in established formulae, then perhaps the vast natural pinyon resources of the West could be developed for some economic advantage.

This study was meant to serve as an introduction into the nature of pinyon oleoresin, and to the problems involved when this substance was used in pharmaceutical preparations. It was anticipated that many questions will be raised by this work, and that it may serve as a stimulus for further studies regarding these possibilities.

## II. REPORTED USES OF PINYON OLEORESIN

Attention was focused on the use of pinyon "gum" remedies as a result of continued reference to it in a general survey which was done regarding pioneer medication. Rumors of its current use for various illnesses also cropped up; and it was deemed valuable to run these rumors down to their source, one by one, to ascertain their reliability. As a result of this, personal interviews were held with a number of the people who were reported to have used pinyon "gum" medicinally.

### A. Internal Uses

Mr. James H. Allen of Cedar Fort, Utah, reported that his father had used pinyon "gum" as a "healing" salve on many types of external abrasions, some of which were infected. Mr. Allen stated that after he had collected some "gum", a local resident asked if he might have some with which to treat an unspecified intestinal malfunction. Mr. Allen was so impressed by the relief described to him, that he decided to try it internally on himself. When he did so, he found it to be very effective, and he has since used it for "worry pains" in the stomach; excess gas; hyperacidity; and as a sure, non-griping, stool softening laxative which was described as bringing relief in a

few hours. Mr. Allen reported that his brother had used the "gum" for "heartburn" with complete satisfaction.

Mrs. Nelda Steadman of Draper, Utah, reported using pinyon "gum" internally as a stool softener, antiseptic, and healing agent for hemorrhoids with complete relief and prevention of onset of irritation.

Mrs. Afton Stauffer of Murray, Utah, reported having a stomach disorder of such a severe nature that her diet consisted only of one-half bowlful of a finely ground cooked cereal to which milk and cream were added. After being persuaded to try pine "gum", she took four doses the first day, five the second day, decreasing to two and then to one a day as she improved. Within two weeks Mrs. Stauffer could eat foods which had been withheld for several years. Mrs. Stauffer reported, also, that she had been treated by a physician prior to using the pine "gum" but without success.

Mr. Delt Fox, a sheepherder from Lehi, Utah, had becom addicted to alcohol to the extent that he refused food if anything with an alcoholic content was available. Mr. Fox reported that he began having severe stomach pains. A physician subsequently concluded that his stomach had been "ruined", and therefore put him on a strict diet. After an unspecified period of time, he

tried some pine "gum" which he said relieved his pain immediately, and after one week of treatment he was able to eat whatever he was served.

Mr. Joseph A. Cutler of Draper, Utah, reported having used pinyon "gum" externally with success, and so decided to try it internally. He stated that he had an intestinal disturbance which caused constant diarrhea and abdominal pain. He had been under a physician's care, and had been given both sulfa and antibiotic therapy without relief. His weight had decreased fifty to sixty pounds, and he had become too weak to work. He reported that within twenty minutes after taking two balls of "gum", the abdominal pains had ceased. That night Mr. Cutler swallowed another ball of "gum" and went to bed. He reported that for the first time in weeks, his sleep was undisturbed. From that time on he began to gain weight, and was able to return to his plumbing work in about six weeks.

Mrs. Blanche Finley of Provo, Utah, reported having had stomach ulcers for years. Being a nurse, she had used many different gastro-intestinal antacids, demulcents, and other drugs without any significant relief. After using crude pinyon "gum", Mrs. Finley said she no longer needed to resort to other medication, and takes pine "gum" only occasionally.

Mr. Earl Hall and Mrs. Afton Hall of Cedar Valley, Utah, reported having used the "gum" for "stomach trouble" with good results.

Mr. Peter Serrano of Murray, Utah, described a condition as being ulcers, and reported a successful treatment with pine "gum".

Mr. Emmett Douglas and Mr. William Jones of Billings, Montana, reported the successful use of pinyon "gum" in the treatment of hemorrhoids, ulcers and other ill-defined gastro-intestinal disorders.

B. External Uses

The attributes of pinyon "gum" for external treatment of various types of wounds are well known to the older natives of central and southern Utah. The pioneers used it on external infections, and on any and all external wounds. They ascribe remarkable healing, cleaning, antiseptic, and "drawing" properties to the oleoresin from pinyon. Indians, as well as white settlers, are reported as having used pinyon as a poultice for toothaches.

Mr. Cyril Stout of Hurricane, Utah, has reported using pinyon "gum" on boils with pain relief in eight hours, and

removal of the core (through the use of the "gum") in one-half to two days. Mr. Stout also stated that he has used pinyon "gum for external infections since his childhood, and always with good results.

Mrs. Marlynn Pollard reported the removal of a sliver of wood, imbedded deeply in the buttocks. After several unsuccessful attempts by lay persons, two applications of the "gum removed the splinter and all associated infection.

Mr. James H. Allen of Cedar Fort, Utah, reported the use of pine "gum" externally in veterinary work. He also recalled that his aunt had used it in treating a local physician's wife who had a carbuncle, which her husband had been unable to cure.

Mrs. Anna Smith of Lehi, Utah, used pine "gum" internally for stomach pains. She also mixed the gum with mutton tallow and beeswax to prepare an ointment.

Mr. Larry C. Stout, a pharmacist in Salt Lake City, Utah, used pinyon in the form of an ointment for hands which had become burned with washing detergents. He reported that the soreness and inflamation were healed in a brief period of time. Mr. Britt Clark and Mrs. Donna Butterfield, of Salt Lake City, Utah, have also reported the satisfactory treatment

of detergent burns with an ointment containing pinyon oleoresin.

Mr. Joseph Cutler of Draper, Utah, reported the external use of pinyon "gum" in treating a case of blood poisoning resulting from a scratched leg. He stated that he was hospitalized for treatment, but without success. Mr. Cutler stated categorically that he believes pine "gum" will cure any external infection.

Mrs. Mattie Sorenson of Draper, Utah, reported that after having stepped on a nail, she developed blood poisoning. She stated that she treated it successfully using only pinyon "gum".

Mr. Don Dunyon of Draper, Utah, had boils over a considerable portion of his body. These boils became healed after the application of pine "gum".

Elray Christiansen of Salt Lake City, Utah, reported having had blood poisoning in his arm. This infection was severe enough to require hospitalization. However, when told by the physician that his arm might have to be amputated, he resorted to the application of raw pinyon oleoresin. After an unspecified term of treatment, the limb again became healthy.

Mrs. Virginia Tanner, a nurse from Salt Lake City, Utah, reported the successful removal of several fragments of glass which had become imbedded in her forehead as the result of an automobile accident. She was given the "gum" in the form of an ointment, by Mr. John Hunter, a pharmacy student at the University of Utah.

# III. GENUS PINUS

## A. Introduction

The pinyon pines of western North America form a distinctive section of the genus Pinus. Because of the apparent utility of the oleoresin from this tree, it seemed important to consider the taxonomic characters delimiting the genus and species. Histological sections of leaves, bark, and wood were included to show the relative size, number and distribution of the resin ducts. The comparative anatomy of the three forms was also demonstrated.

Species of pines are widely distributed throughout the northern hemisphere from the Arctic Circle to the West Indies, the mountains of Central America, the Canary Islands, northern Africa, the Philippine Islands, and Sumatra. The richest development of genera and species is in western North America and the temperate parts of eastern and central Asia (Raphael, 1923).

Pinus are seed plants belonging to the class Gymnospermae. The criteria delimiting this genus are that its members are resinous trees or shrubs having pollen or seed bearing structures termed strobili or cones. Cotyledons number from six to ten, or as many as seventeen. The leaves are terete,

channeled, or flat occurring singly or in clusters with a membranous sheath surrounding the base. Leaves are usually straight veined and persistant (Benson, 1957).

The pines include trees from which the majority of the world's timber is obtained. Many chemicals, cellulose products, protectives, and medicaments also come from these trees. Food in the form of seeds or nuts is derived from the Pinyon pine.

## B. Pinyon Pines

The name piñon was given to these trees by the early Spanish explorers. They were first described by Nunez Cabeza de Vaca, a scholarly nobleman, in 1535. Piñon is a Spanish word meaning pine nut. Its spelling has been Anglicized to pinyon.

There are four species of nut pine or pinyon. The chief characteristic by which they are defined is the manner in which the leaves are borne. Pinus quadrifolia Parry (Parry pinyon) has needles in clusters of four, Pinus cembroides Zucc. (Mexican pinyon) usually in clusters of three, Pinus monophylla Torr. and Frem. have needles which occur singly, Pinus edulis Engelm. (Colorado pinyon) has needles in pairs

or occasionally in clusters of three (Collingwood, 1937).

It has been estimated that there are approximately twenty-five million acres of pinyon-juniper forest in the south-western states, or seventeen per cent of the land in Arizona and New Mexico. They are also found widespread in Nevada, Utah, Colorado, south-western Texas, and northern Mexico (Figure 1). In general, pinyon are found between five thousand and seventy-five hundred feet elevation where precipitation ranges from twelve to fifteen inches per year, and the temperature from one hundred ten to minus twenty-five degrees Fahrenheit. Pinyon are usually associated with Quercus Gambelii Nutt. (Gambel, Scrub, Utah, Shin, or Mountain Oak), Taxus brevifolia Nutt (Pacific Yew Mountain Mahogany), Juniperus monosperma Engelm. (Oneseed Juniper, New Mexico Cedar), Juniperus Deppeana Steud. (Mountain, Thickbark, Oak-barked, or Checker-barked Cedar), and Juniperus osteosperma Torr. (Little Desert Cedar, Utah Juniper) (Sargent, 1926). Pinyon are found growing on gravelly or rocky soils; and, in spite of their shallow root system, they seem to be very wind resistant.

Pinyon trees are seldom injured by fire, but excessive grazing may destroy the seedlings. The worst enemy is probably

Figure 1[1]

Range of the Pinyon Pine

[1] Photographed from Rocky Mountain Trees, Preston, 2nd edit


**RANGE OF THE PINYON PINE**

the "pinyon blister rust", a two-host fungus disease similar to the white pine blister rust. As in the case of the white pine rust, the alternate host of the pinyon rust is a wild currant, and the damage is chiefly sustained by seedlings and younger trees (Collingwood, 1937).

The natural reproduction of pinyon is limited because seed years only occur every second to every fifth year. Other factors which limit the propagation of the species are: unfavorable climatic conditions, non-viability of seeds, the rapidity with which seeds lose their germination power, loss of seeds (eaten by rodents, birds, and men), unfavorable soil, and overgrazing (Phillips, 1909).

1. Pinus edulis Engelm. [Pinus cembroides var. edulis (Engelm) Voss] (Piñon, Nut Pine, Piñon Pine, New Mexican Piñon, Scrub Pine, Pitch Pine, Colorado Pinyon, Mesa Pinyon, Pinyon, Two-leaved Common Pinyon).

Sargent (1897) credits F. A. Wislizenus with the discovery of Pinus edulis; however, this may have been the same species which was originally described by the Spaniard Cabeza de Vaca in 1535 (Collingwood, 1937). Pinus edulis is the state tree of New Mexico.

The key characteristics of the two-leaved pinyon are given by Sargent (1897): "Leaves in 2 or 3-leaved clusters, stout, rigid, sharp-pointed, from 3/4 of an inch to 1 1/2 inches in length. Cones from 1 1/4 to 1 1/2 inches long."

Pinus edulis rarely grows more than forty feet tall, frequently with a short, divided trunk and a low, round-topped head of spreading branches. Its outline is conical when young, becoming more round and flat as the plant becomes older. Thick branchlets are orange-colored during their first and second years, finally becoming light gray or dark brown, sometimes tinged with red. The needles are sharp-pointed, often curved, with smooth margins. Those of seedlings and of new growth are a bright bluish green. They remain on the branches as long as nine years, but begin to fall with the fourth season (Rogers, 1926). The surface of the needles is marked with from five to fifteen parallel rows of stomata. In cross section, two peripheral resin ducts and a single central vascular bundle can be seen (Cole, 1956).

Clusters of elongated, dark red, pollen-bearing cones cover the tree in early spring. Short stalked, purplish, ovulate strobili are found on the ends of the twigs at the same time. The pollen cones soon drop, but the ovulate ones

## Plate 1 [1]

Pinus edulis Engelm

1. A branch with pollen cones.
2. A pollen cone, enlarged.
3. Bract of a pollen cone, enlarged.
4. Diagram of the involucre of a pollen cone
5. A microsporophyll, front view, enlarged.
6. A microsporophyll, rear view, enlarged.
7. A branch with ovulate cones.
8. An ovulate cone, enlarged.
9. A scale of an ovulate cone, lower side with its bract, enlarged.
10. A scale of an ovulate cone, upper side, with its ovule enlarged.
11. A branch with mature ovulate cones.
12. A cone scale, upper side, with its seeds.
13. Vertical section of a seed, enlarged.
14. An embryo, enlarged.
15. A cluster of leaves with its sheath.
16. Tip of a leaf, enlarged.
17. Cross section of a leaf, magnified fifteen diameters.
18. Winter branch buds.

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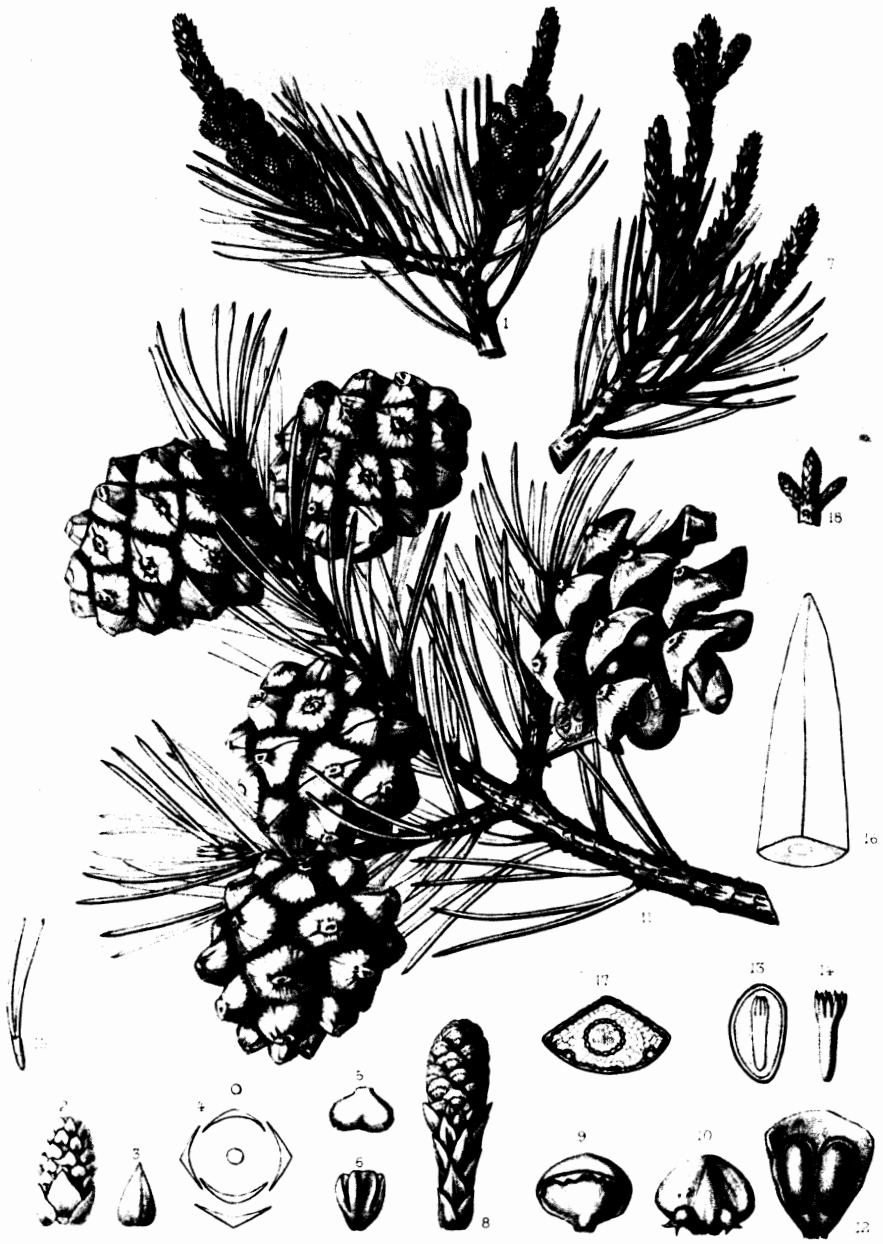
[1] Photographed from Sargent, C. S., Silva of North America Volume XI, Plate 552 Peter Smith, New York, 1947 (Reprinted from 1897 original).



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PINUS EDULIS. Engelm.

A Riocreux direx.t Imp. J. Taneur. Paris.

develop during August and September of the second year into egg-shaped, shiney, yellowish brown cones about one inch to two inches long. The cone scales are relatively few in number and without prickles. Two to thirty red-brown, mottled, nut-like seeds are on the scales near the middle of each cone. The bark is from one-half to three-quarters of an inch thick and irregularly divided into connected ridges covered by small, light brown scales tinged with red or orange color. Wood is light, soft, not strong, brittle, pale brown, largely used for fuel and fencing. In the past it has been used as a source of charcoal for smelting.

Jones (1891) considered Pinus edulis to be a variety of Pinus monophylla because single and paired leaves had been frequently found on the same individual tree. Sargent (1897) stated that he could find none that did not fit the description for Pinus monophylla even though the two species are connected by trees in southern Utah that have both single and paired leaves in about equal numbers. A difference in the morphology and color of the leaves (Pinus edulis are usually darker, shorter, and less spinescent than those of Pinus monophylla ) led Sargent (1897) to make a species distinction between the two forms.

2. Pinus monophylla Torr. and Frem. [Pinus edulis var. monophylla Torr., Pinus cembroides var. monophylla (Torr. and Frem.) Voss.] (Piñon, Nut Pine, Gray Pine, Nevada Nut Pine, Single-leaf, Fremont's Nut Pine, Single-leaf Piñon, Single-leaf Pine)

Pinus monophylla was discovered in 1842 by Fremont and Torrey during an expedition which was commissioned to find a pass through the Sierra Nevada mountains to the interior valleys of California. The tree was originally named Pinus monophyllus by Dr. Torrey. Captain Fremont recorded in his diary that the seeds or nuts from this tree were a welcome addition to their then dwindling food supply (Fremont, 1845). Pinus monophylla is the state tree of Nevada.

The key characteristics of the single leaf pinyon are given by Sargent (1897) as follows: "Leaves solitary or rarely in 2-leaved clusters, stout rigid, spinescent, from 1 1/4 to 2 1/4 inches in length. Cones from 1 1/2 to 2 1/2 inches long."

Pinus monophylla grow to an average height of from fifteen to twenty feet. Occasionally they may reach forty to fifty feet in height. The trunk is very short, often divided near the ground into several strong spreading branches; these

are short and stout, forming a compact conic head. The older trees usually have longer more pendulous branches, and are more round topped or irregular. The bark of the trunk is about three quarters of an inch thick, and is divided by deep irregular fissures. The leaves are solitary and terete, or occasionally in two-leaved clusters and semi-terete; they are rigid, incurved, entire, spinescent with long callous tips, pale glaucous green, and usually about an inch and a half long. They are marked with from eighteen to twenty-six rows of stomata, and contain two or three resin ducts and a single fibro-vascular bundle. The pollen strobili are about a quarter of an inch long, oval in shape, and dark red in color. The ovulate strobili are oval with thick rounded scales. These scales are covered by ovate lanceolate light chestnut-brown bracts. In autumn the young cones are oblong, erect, and about half an inch long. Beginning to grow very early the following spring, they are nearly half grown when the cones open in May. At maturity they are from one and one-half to two and one-half inches in length, somewhat less in breadth.

## Plate 2

### *Pinus monophylla*[1]

1. A branch with pollen cones.
2. A pollen cone, enlarged.
3. Diagram of the involucre of a pollen cone.
4. A microsporophyll, front view, enlarged.
5. A microsporophyll, side view, enlarged.
6. An end of a branch with ovulate cones.
7. An ovulate cone, enlarged.
8. A scale of an ovulate cone, upper side, with its ovules, enlarged.
9. A branch with mature ovulate cones.
10. A cone scale, upper side, with its seeds.
11. A cone scale, under side.
12. Vertical section of a seed, enlarged.
13. An embryo, enlarged.
14. A two-leaved cluster of leaves.
15. Tip of a leaf, enlarged.
16. Cross section of a leaf, magnified fifteen diameters.

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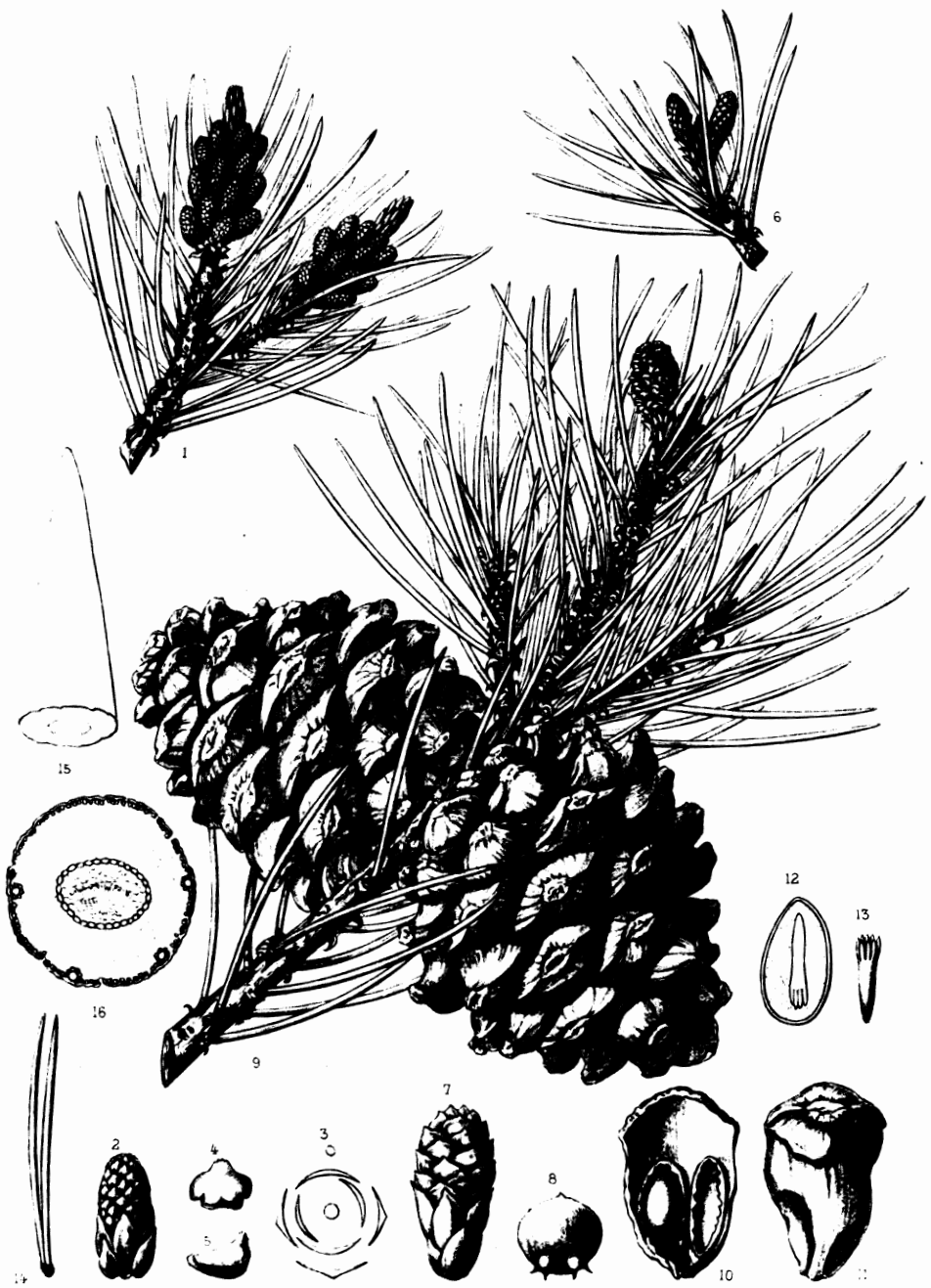
[1] Photographed from Sargent, C. S., Silva of North America, Volume XI, Plate 551. Peter Smith, New York, 1947 (Reprinted from 1897 original).

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PINUS MONOPHYLLA, Torr.

A. Riocreux direx.t Imp.r J. Tinneur, Paris.

3. Anatomy

The histological descriptions of leaves, bark, and wood are described by Cole (1956).

(a) Leaves. Cross sections of the needles of Pinus edulis showed a characteristic channelled outline. They contained but a single fibrovascular bundle and usually two resin ducts that were found to be somewhat toward the ventral surface and peripheral. The epidermis was two cells thick with uniformly thickened cell walls, the external surfaces of which were heavily cutinized. The mesophyll showed curiously involuted mesophyll cells, many of which were filled with resinous material that was deeply stained by the safranin. In the central or stelar region the conductive tissue was seen, surrounded by transfusion tissue, and separated from the mesophyll by a rather prominent endodermis composed of thin walled cells. The resin canals were found to be lined with epithelial cells.

Cross sections of the leaves of P. monophylla were found to be the same as those of P. edulis except that they were circular in outline and exhibited a larger number of resin ducts on the average.

Cross sections were also cut of the leaves of an intermediate form of tree that had both terete and chanelled needles in about equal numbers. These sections were examined and those that were cut from needles of a shape similar to either the channelled P. edulis or the terete P. monophylla showed identical characteristics with their morphologic equals.

These findings were in agreement with those of Harlow (1931). The characteristics of all three forms may be seen in Plate 3.

(b) Bark. The transection is surrounded by a layer of bark on the outside. The bark contains a layer of

Plate 3

Leaves[1]

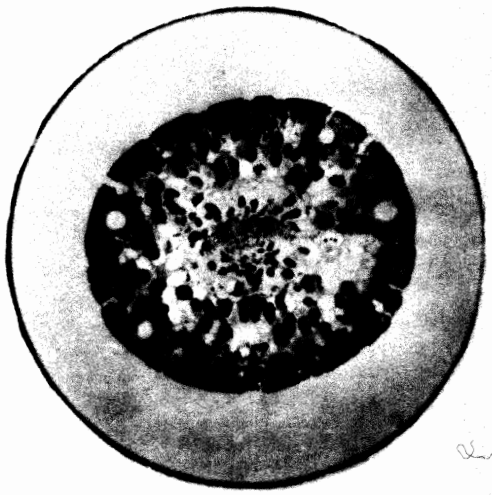
a. Transverse section of *Pinus monophylla* leaf, 80X

b. Transverse section of *Pinus edulis* leaf, 80X

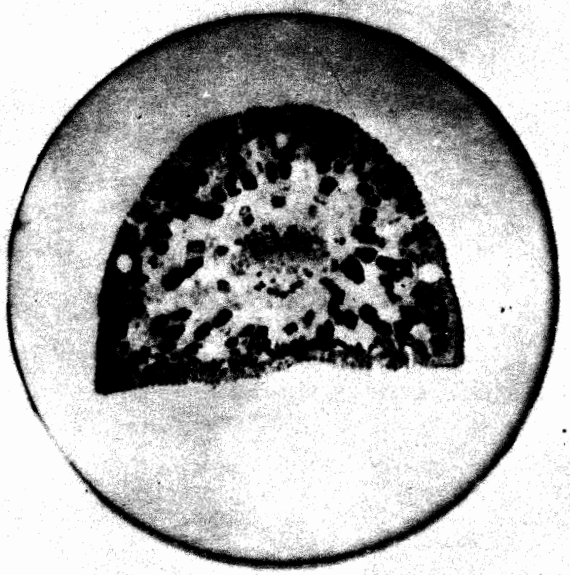
c. Transverse section of Terete leaf of intermediate, 80X

d. Transverse section of channelled leaf of intermediate, 80X

[1] Photographed from Cole, F. R., The Pharmacognosy of U
Pinyon Pines, Plate 2, Ph. D. Thesis. Salt Lake City, Utc
University of Utah. 1956.



a.



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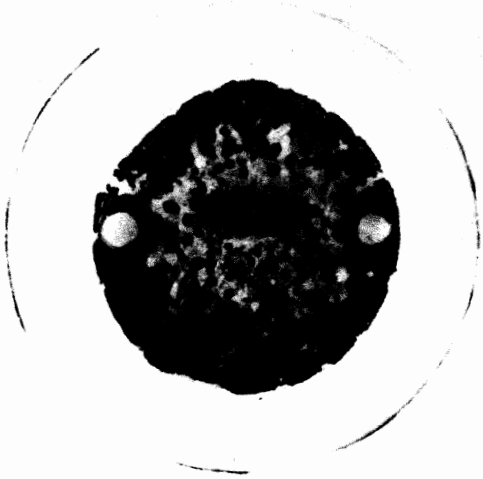
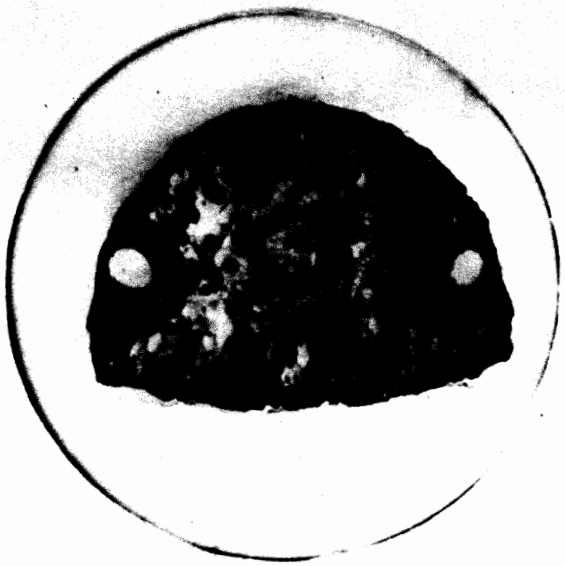


c.



d.

cambium and secondary phloem cells next to the wood that is composed of more or less wavy rows of cells and interspersed with round resin containing parenchyma cells. The phloem area occupies about 30 per cent of the total thickness of the bark.

The outer bark and cortex occupy about 70 per cent of the total thickness of the tissue. The rough corky outer layers of the phellem are in straight radial rows and more or less crushed in appearance. These cells form the fissured outer surface of the stem and are in a layer that is usually 8 to 10 or more cells thick. The phellogen layer is approximately 4 to 6 cells thick. These cells have uniformly thickened walls and are in straight radial rows.

The cortical layers of the bark comprise about 50 per cent of its thickness. The cortex is composed of thin walled ordinary parenchyma cells some of which are filled with a deep staining resinous material. Large resin ducts lined with epithelial parenchyma are found in the cortical region. The resin ducts of the cortex are several times as large as those found in the xylem and much more numerous.

The bark of the three types was observed to be nearly identical in its anatomy. However, the bark of P. monophylla contained resin ducts that were somewhat larger than the others.

Plate 4 illustrates the bark characteristics described above.

(c) Wood. The wood is rather uniform in character, with fairly pronounced growth rings and conspicuous wood rays that are one cell wide. Numerous resin ducts were noted, each duct being lined with epithelial parenchyma. The tracheids are thick walled and heavily lignified.

The stems of the intermediate form of pine were cut in cross section and examined. The xylem of all

Plate 4

Bark [1]

a. Transverse section of bark of *Pinus monophylla*, 80X

b. Transverse section of bark of *Pinus edulis*, 80X

c. Transverse section of bark from the intermediate form, 80X

[1] Photographed from Cole, F. R., The Pharmacognosy of Utah Pinyon Pines, Plate 5, Ph. D. Thesis. Salt Lake City Utah. University of Utah. 1956.

a.

b.

c.

three forms, P. edulis, P. monophylla, and the intermediate appeared to be identical.

Typical xylem arrangement of the three forms is shown in Plate 5.

Plate 5

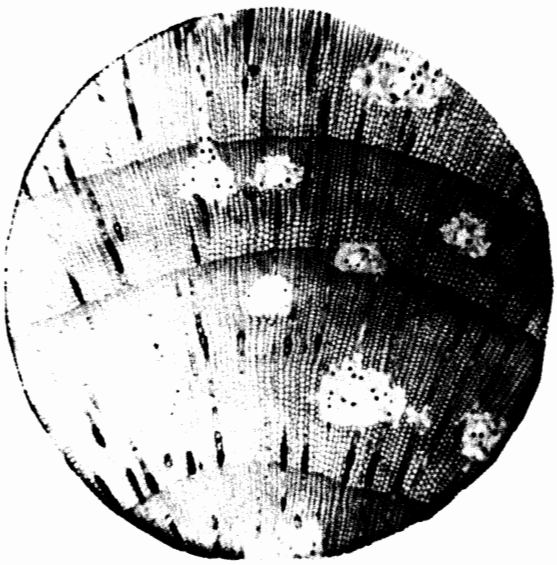
Wood [1]

a. Transverse section of *Pinus monophylla* wood, 80X

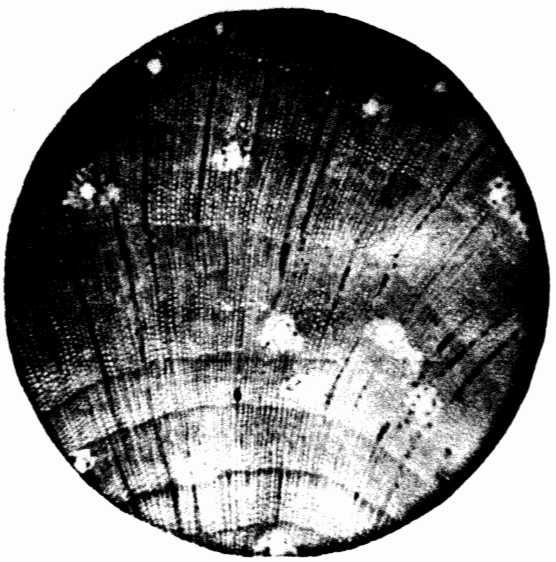
b. Transverse section of *Pinus edulis* wood, 80X

c. Transverse section of wood from intermediate form, 80X

[1] Photographed from Cole, F. R., The Pharmacognosy of Uta
Pinyon Pines, Plate 4, Ph. D. Thesis. Salt Lake City, Utal
University of Utah. 1956.



a.



b.

c.

# IV. COLLECTION OF THE OLEORESIN

The collection of pinyon oleoresin in amounts sufficient to make their utilization commercially feasible has met with little or no success. It has been shown, in the case of pinyon, that subjection of the trees to the conventional scarification method of extraction used in turpentining will yield amounts of oleoresin slightly over one-half of that obtained from the south-eastern pines. Therefore, pinyon could not compete for the "naval stores" market. Whether or not a particular method of collection, and the yields therefrom, are of practical significance would depend on the demand created as a result of some unique quality possessed by the product.

The medicinal uses of pinyon oleoresin previously cited indicate that the amount of oleoresin necessary to manufacture the various pharmaceutical preparations might still be satisfied by the limited yield from pinyon pines. The problem, then, was to produce an oleoresin of sufficient purity, and in sufficient quantity to make its utilization in the preparation of pharmaceuticals practical.

With this in mind, consideration was directed toward various methods of collection, the amount of impurities involved, and the optimum growing conditions for maximum yield from the trees.

Deaver and Haskel (1955) reported that the minimum number of potentially commercial pinyon for oleoresin should average twenty to twenty-five trees per acre, and that they should be over six inches in diameter at breast height. They also reported that when tree scarification was employed the yield of oleoresin tended to increase with the advance of the seasons, but due to the variability of trees studied, this increase was shown not to be significant. It was also observed that the elevation of the area where the trees are located might be a factor. With an increase in elevation, there tended to be a decrease in average yield until the transition zone between pinyon and ponderosa pine was reached. However, there was no significant variation in resin yield in considering the limits of error on the measure of variability of the samples.

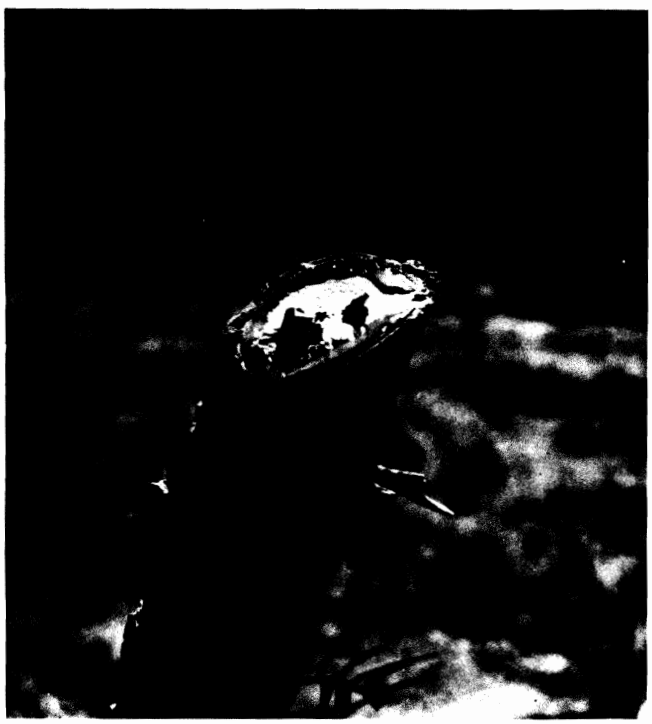
Several long-time users of pinyon oleoresin had reported that some of their collections of crude material caused nausea. Upon further investigation, it was found that in

these cases the pinyon "gum" was taken from Pinus edulis. The naturally occurring oleoresin produced by the intermediate variety described by Cole (1956) produced no such nauseating symptoms, and is apparently the product which had been utilized in all medicinal reports cited. For this reason, the collections for this research were restricted to this hybrid.

This study was begun in June of 1959. The area chosen for the field investigations was near Sabie Mountain on the Wasatch National Forest in Tooele County, Utah. The initial work was for the purpose of determining the depths and types of cuts to be used, and also the amounts of exudates which might be expected during subsequent collection periods, using different methods.

A hatchet was used to remove the outer bark, and a linoleum or banana knife was used for the scarification of the trees (Plate 6-a). A limb of two and one-half inches in diameter was cut from a tree using large pruning shears. The cross section thus made was sufficiently "clean" to allow observation of the relative position and concentration of resin ducts as evidenced by the exudate formed. It was seen that the majority of these ducts were located adjacent to, and inside of the cambium layer. The cross section in Plate 6-b

a



b

Plate 6

shows the amount of oleoresin which had exuded after ten minutes. The required depth of future scarification of the tree trunk was indicated by the relative point of exudation.

Vigorous, healthy pinyon showing both single and double needle fasicles, of at least six inches in diameter four and one-half feet above the ground (DBH) were selected. Trees were chosen which were fairly straight and free of branches for three to four feet above the ground. Those that were gnarled, twisted, or those containing partially dead branches were not used.

## A. Chip Method

At a level of from twelve to eighteen inches above the ground, the dead bark was removed with a hatchet. The cleaned area did not exceed one-third of the circumference of the tree. An incision was made one-half inch deep and parallel to the ground at the lower part of the cleaned area. An aluminum gutter was placed in this horizontal incision and bent to direct the flow of the oleoresin.

A broad, horizontal, V-shaped notch was cut one-half to three-fourths of an inch deep in the wood. This was directly over the aluminum gutter which directed the

oleoresin into a can, four and one-half inches deep and three and nine-tenths inches in diameter. This can was placed directly under the gutter and was supported by a nail driven into the tree at the top of the can. The can was held securely by the gutter and the nail as shown in Plate 7-a.

Only vigorous, healthy pinyon were selected as collection stations. Trees which had any evidence of insect infestation or disease were rejected. In this case, eight trees were used. After placement of the cup and gutter, the oleoresin was collected for a four week period. It was noted that most of the material was collected in the first two weeks. Thereafter, flow was restricted as the oleoresin became less liquid, and subsequently obliterated the resin ducts.

The total yield was determined by weighing the collection can with its contents, and then subtracting the weight of the can alone. The total yield for this period was 203.4 grams. Not included was the collection from station number three, part of which was lost. Collections ranged from 21.6 grams to 34.5 grams per station.

a

b

Plate 7

## B. Scrape Method

The medicinal uses of pinyon oleoresin reported previously have all been concerned with the natural exudate found on the trees. The term usually applied to this product is "pinyon gum" or "pine gum"; however, this terminology refers to the sticky, chewey nature of the oleoresin rather than to the presence of any true gums (Mirov, 1956).

"Pinyon gum" forms as a result of some natural injury. The most common is that inflicted by the porcupine, as it strips off the dead outer bark in order to gain access to the softer, more succulent edible tissues underneath.

A considerable amount of oleoresin may accumulate at the place of injury. Several ounces of oleoresin may be removed from a single site, the amount depending on the extent of the injury, location of the injury, climatic conditions, and length of time the tree has been injured (Plate 7-b). When the oleoresin exudes, it becomes partially oxidized as it accumulates and is exposed to the elements (George, 1954).

With the view to using the exudate from intentionally injured pinyon as a source of natural oleoresin for pharmaceuticals, it seemed advisable to scarify, or otherwise injure,

some sample specimens (Plate 8). Twenty months after treatment these sample trees were again examined. It was concluded that the small amount of oleoresin which had accumulated in this length of time would not be sufficient to satisfy commercial requirements (Plate 9).

After spending some time collecting oleoresin which had accumulated because of some natural injury, it was concluded that this procedure would be inadequate. This conclusion was based on the amount which could be obtained, the length of time required for collection, and the number of years which apparently is required for production of significant amounts of oleoresin after the initial injury. It seemed evident that after the accumulated oleoresin resulting from natural injuries has been exhausted, some other means of obtaining the raw material would have to be devised.

## C. Bore Method

The problem of purity became a consideration when it was found that a considerable amount of foreign matter was mixed with the oleoresin when it was collected by the open methods (chip, scrape, and natural exudate). The presence of this extraneous matter makes the purification of the

Plate 8

Plate 9

oleoresin much more difficult, particularly when the consideration is for its subsequent utilization in pharmaceuticals intended for human use.

In an attempt to develop a system of collection which would result in an uncontaminated product, and also one which would produce a good yield, a closed method was devised. This system will be designated as the "Bore" method.

Holes were drilled into vigorous, mature pinyon. The diameter of these holes was adjusted so that a thirteen dram, wide mouth, plastic vial could be fitted securely into it. The holes were made from one and one-half to two inches deep, depending on the thickness of the bark. In the pilot study, holes were drilled at various angles to the trunk and limbs of the trees (Plate 10). The best angle and placement of the vials was found to be on nearly verticle trunks at an angle of approximately $45^0$. The vials were placed so that the upper edge of the mouth of the vial was just inside the outer surface of the bark so as to allow an unobstructed flow of oleoresin. At the same time, foreign matter was prevented from gaining entrance into the collection vial.

Following the pilot study, twelve collection vials were placed as described. The first collection period was

Plate 10

from June 6, 1959 to July 24, 1959; the second from August 9, 1959 to September 6, 1959; and the third from November 25, 1959 to March 10, 1960. Table I gives the amounts of oleoresin collected in the respective collection periods. At the beginning of each period, new vials were placed into the twelve bore holes. The total amount of oleoresin collected was seventy-six and two-tenths grams. It was a clear, colorless, viscous liquid which, upon standing, developed numerous white, opalescent crystals. These crystals were assumed to be those of abeitic and resinic acids.

Table 1

| Method | Grams Collected (Average per station) | | |
|---|---|---|---|
| | From 6-6-59 to 7-24-59 | From 8-9-59 to 9-6-59 | From 11-25-59 to 3-10-60 |
| "Bore" | 16.0 | 21.2 | 7.2 |
| "Chip" | 10.4 | 29.1 | --[1] |

[1]Collection could not be determined.

Apparently there was no permanent damage to trees subjected to either the scarification or the bore and vial procedures. This was determined by comparing the general

appearance of the trees used for collection with those of the same size in the same plot which were not used. The appearance of dead branches or leaves, and evidence of discoloration were taken as signs of injury. The comparison was made after a twenty month period.

Deaver and Haskell (1955) reported little or no effect on the vigor of the pinyon utilized in their study. Generally, they found that the number showing a loss of vigor to be nearly equal between chipped and un-chipped trees. Inspection by these same workers of several trees which were chipped by Colton, fourteen years earlier, showed them to be still in vigorous and healthy condition.

Even though the "bore" method of collection produced a more pure, unoxidized oleoresin, it was felt that the yield was not adequate to meet any projected commercial demands.

# V. REFINEMENT

Reports for this study indicate that until the present time pinyon oleoresin used for medicinal purposes has been applied or consumed in its crude form without any particular regard for purity. In this state the oleoresin is usually contaminated with all manner of debris. Insects, pollen, bits of wood and bark, needles, dirt, etc. may have become embedded in the excrescence as it accumulated on the tree. Mirov (1953) reported that this material, mixed with soil and pine needles, produced a low-grade product because of these impurities. Upon distillation of the oil from the resin, he concluded that an impure turpentine and solid resin of no commercial value was produced.

Since the bore collection was almost completely free of contaminants, it was considered unnecessary to subject it to any refinement prior to use.

The product from the chip collection was relatively free from trash. However, there was some foreign material present. Because of these impurities, the direct utilization of the oleoresin into pharmaceutical preparations was not advisable.

Throughout all of the refining processes in this study, care was exercised to avoid any alteration of either the chemical or physical nature of the product. Even so, the semisolid state of the natural exudate and the cup and gutter collection, together with the undesirable trash present necessitated the utilization of either heat or solvents before the oleoresin could be considered suitable for use.

The possibility of forming new chemical compounds or complexes, and thus altering the pharmacological activity of the parent product, precluded the use of solvents for the purpose of liquifying the raw material for filtration.

Before the oleoresin could be liquified by heat for screening or filtration, it was necessary to determine if any loss by volatilization would occur at a temperature sufficiently high to liquify the raw oleoresin. To accomplish this, a charge of crude material was placed in a three neck, angle-type distilling flask equipped with a Cleavenger (1928) apparatus designed for collection of volatile oils which are lighter than water. The distilling flask was also equipped with a centigrade thermometer. Heat was obtained from a heating mantle (Plate 11).

Screening of the oleoresin samples was possible after heating to 76$^0$C. At this temperature the sample could be easily poured into the distillation flask. There was no evidence of volatilization at this point. The first evidence of any condensed oil in the Cleavenger trap was at 136$^0$C, measured at atmospheric pressure. At this temperature the oleoresin could be easily manipulated, but without the loss of any volatile components.

The first attempt at refinement was to pour the oleoresin, heated to 130$^0$C, onto a number sixteen mesh wire screen. This method proved to be very slow, as the liquid oleoresin became very viscous as it came in contact with the unheated wire. Therefore, before straining could proceed, it was necessary to periodically heat the wire mesh over a hot plate so that the accumulated debris and solidified oleoresin could be removed. After several trials, this procedure was abandoned as being too slow and laborious to be of any practical significance. It was apparent that a straining method could be successful only if the screening material were maintained at a temperature at least as high as the liquified oleoresin being refined.

To accomplish this, it was thought feasible to make use of an electrical device in which heat could be produced by attaching the positive and negative terminals of a 110 volt, 60 cycle, alternating current to opposite sides of a resistant wire mesh. The voltage was made adjustable by a variable transformer. Using common iron screening, it was not possible to produce an even heat over the entire surface of the screen. Heat was most intense at the point of connection of the wire.

To circumvent this difficulty, a stainless steel wire mesh was employed to which a plate had been brazed across opposite edges. A connecting terminal was in turn welded to these plates. When the alternating current previously described was impressed upon the device, it was heated only slightly and insufficiently at very low voltages. As the voltage was increased, in an effort to raise the temperature, it was found that the required resistance was too great, and thus the circuitry became overloaded.

Even though these attempts with alternating current were unsuccessful, an industrial engineer might find a satisfactory solution to this problem by either enclosing the coils

to prevent a short circuit, or perhaps some application of a direct current might be feasible.

To assure that the screening gauze would be at least as hot as the oleoresin, it was bent to conform as nearly as possible to the shape and size of the heating vessel (Plate 11). The raw material was then placed in the wire gauze. The temperature was kept below the volatilization point by the use of a steam bath to heat the screening device. After the crude material had become sufficiently liquid, the wire gauze could be raised up through it, with the subsequent removal of the objectionable trash. This method of refinement has been reasonably successful as a pilot study, and it is felt that it could be adapted for production on a large scale.

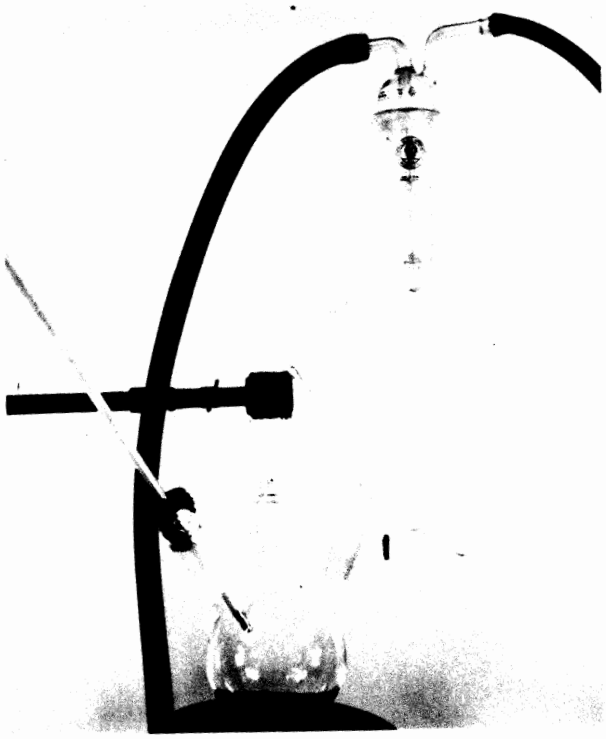


Plate 11

# VI. ANALYSIS

## A. Analysis of the Oil

### 1. Assay for Oil Content

The oil fractions from samples of the three collection methods ("scrape", "chip", and "bore") were all assayed and purified in the same manner.

A sample of oleoresin from each of the collection methods was assayed for volatile oil content by the N.F. XI method. The crude plant exudate was first strained, and then placed in a three neck, angle-type distilling flask equipped with an apparatus (Cleavenger, 1928) designed for collection of volatile oils which are lighter than water (Plate 11). The calibrated side-arm of the trap was filled with water so that the condensed oil did not have to rise through the tube, thus eliminating the possibility of obtaining a false reading. A measured quantity of oleoresin was poured into the distilling flask and covered with water. The volume of oil entrapped in the calibrated side-arm was read and the percent oil content calculated on the basis of volume of oleoresin used in each assay. The results of these assays are shown in Table 2.

Table 2

| Method of Collection | Sample Volume (ml.) | Oil Volume (ml.) | Percent Yield |
|---|---|---|---|
| "Scrape" | 85.0 | 9.0 | 11.53 |
| "Chip" | 100.0 | 14.8 | 14.80 |
| "Bore" | 70.0 | 13.2 | 18.85 |

The heating mantle was maintained at a suitable temperature ($100^0$ - $105^0$C) to insure gentle boiling of the contents of the flask. Steam carrying the volatile oil rose up through the neck of the flask, both being condensed on the surface of the pear-condenser. The oil, being lighter than water, floated up into the guage glass below the condenser while the water of distillation returned to the retort by way of the inclined side-arm. The return of the oil-saturated distillation water to the retort facilitated a more complete extraction.

Six to eight hours extraction was usually sufficient to remove all of the volatile oil from the oleoresin. Extraction was considered complete when there was no further condensation of volatile oils, as measured on the calibrated column, and when there was no detectable odor coming over from the distilling flask.

Even though hydrodistillation, in general, is not considered as efficient as steam distillation; it did, in this study at least, provide the most convenient method of extraction using the apparatus described above. Guenther (1948) stated that hydrodistillation is especially suitable for powdered materials; however, he also stated that the shape of the retort should be a sphere or a large cylinder rather than a cone since rapid boiling is necessary for maximum efficiency.

Any large scale separation of the oil and resin fractions would probably involve hydrodistillation. In this process larger volumes of steam are not necessary, thus creating an important economic advantage over straight steam distillation in which considerable quantities of steam are necessary since it is run through the plant material only once.

Since the material in the retort must be boiled for several hours, there is the possibility of hydrolyzing the oil constituents. Guenther (1948) points out this disadvantage to hydrodistillation.

## 2. Purification of Oil

The volatile oil collected in the apparatus described above, was cloudy and colorless in all three extractions. The

oils, which were lighter than water, rose to the top of the water in the calibrated side-arm of the trap. The oil, and the water in the bottom of the trap were tapped off into a seporatory funnel. The water was then drawn off, leaving the impure volatile oil.

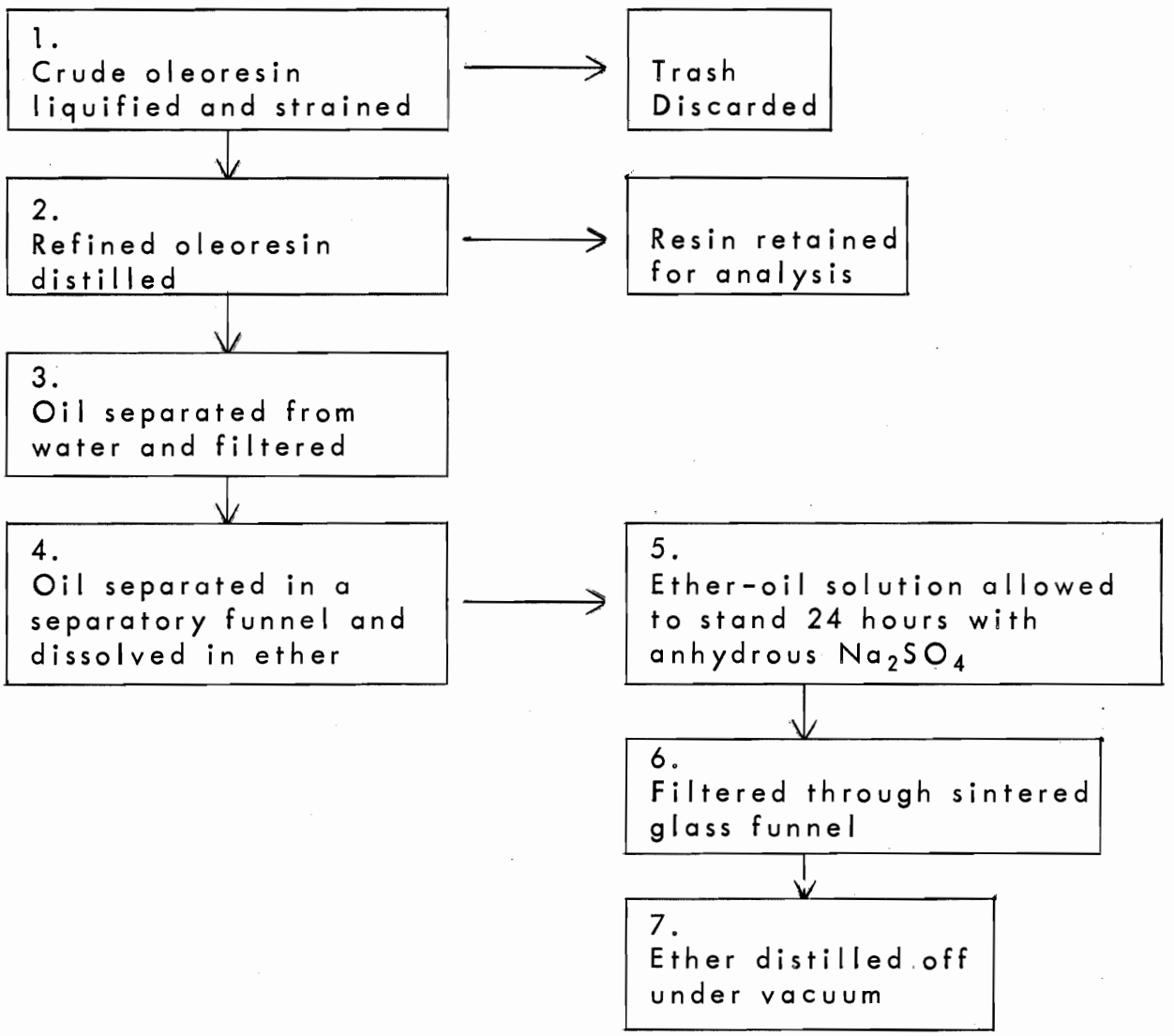
The oil mixture was then filtered through a medium sintered glass funnel to remove any insoluble material. Next, the oil was separated from the water and dissolved in an equal volume of ether. An amount of anhydrous sodium sulfate equal to approximately one-third the volume of the ether-oil solution was added and allowed to stand for twenty-four hours. The sodium sulfate was then removed by filtration through a dry sintered glass filter. The ether was volatilized and removed under a partial vacuum leaving the dry, purified volatile oil. The refinement, extraction, and purification processes described above are illustrated in the flow diagram shown in Figure 2.

3. Yields

The quantity of oil obtained by the distillation of oleoresin from "scrape", chip", and "bore" collections is shown in Table 2 (page 56).

Figure 2

Flow Diagram of Refinement, Extraction and Purification of Volatile Oils From Pinyon Oleoresins



It should be pointed out that these data can be considered as only approximations since the volume of the oleoresin sample used could be determined only by gross measurements using a hundred millileter graduate. In addition, it was impossible to transfer the measured amount quantitatively to the distilling flask because of the viscous consistancy and the tendency of the oleoresin to solidify on the graduate. However, each sample was measured and transferred in an identical manner. Therefore, a comparative evaluation of the different yields should be valid.

Guenther (1955) examined the oleoresin obtained by chipping Pinus edulis Engelm. and reported that when subjected to steam distillation, a volatile oil was separated in twenty-four and nine-tenths percent yield. A report to Deaver and Haskell of the University of Arizona from George (1954) states that when the chipping oleoresin was stripped at three millimeters of pressure to a pot temperature of one hundred and fifty degrees centigrade for removal of the volatile oils, the resin residue amounted to about eighty percent of the oleoresin. An earlier evaluation by Schorger (1913) gives the proportion of volatile oil in the "chip" oleoresin of Pinus edulis as twenty percent.

The differences in yields of volatile oil reported by previous workers as well as the difference between these and the present study may be explained on the basis of species difference, different extraction techniques, or perhaps the loss of volatile constituents during storage before analysis.

## B. Analysis of the Resin

The resin left after distillation of the volatile oils was retained for consideration of some of its physical and chemical properties.

### 1. Acid Number

In accordance with a method of Mantell (1942), one gram of resin from each collection was dissolved and subsequently titrated with a 0.1 N solution of alcoholic potassium hydroxide, using phenolphthalein as the indicator. The acid value reported is the number of milligrams of potassium hydroxide required per gram of resin. The results are shown in Table 3.

Table 3

| Method of Collection | Acid Value |
|---|---|
| "Scrape" | 132.4 |
| "Chip" | 156.8 |
| "Bore" | 163.6 |

2. Saponification Number

Using the method outlined by Mantell (1942) one gram of each resin sample was dissolved and titrated with a standard 0.3 N aqueous solution of sulfuric acid. The saponification number is reported as the milligrams of potassium hydroxide per gram of resin. The results are shown in Table 4.

Table 4

| Method of Collection | Saponification Value |
|---|---|
| "Scrape" | 170.5 |
| "Chip" | 168.0 |
| "Bore" | 173.9 |

3. Fusion Points

Resins do not possess a sharp melting point, but rather undergo a gradual fusion when heated. On heating, two critical temperatures may be observed. The first, known as the drop softening point, is evidenced by softening and blunting of the sharp edges of the resin. The second, termed the melting point, is when actual liquifaction occurs. These critical points are usually designated by a short range of temperature rather than by a finite point.

(a) Drop Softening Point. This method is suitable for resins with a melting point below 125$^0$C. It was described by Krumbhaar (1947) and was carried out as follows: by dipping a thermometer into the fused sample, a uniform layer of resin is spread on the bulb. The temperature at which the resin starts to fall off the bulb in a uniform drop is recorded as the end-point. The drop softening point for the three resins in this study are shown on Table 5.

(b) Melting Point. The determination of the melting points was carried out according to a modification of a method originally described by Durrans (1929). A sample was sintered

Table 5

| Method of Collection | Drop Softening Point |
|---|---|
| "Scrape" | 57 - 58°C |
| "Chip" | 55 - 58°C |
| "Bore" | 55 - 57°C |

to the bottom of a porcelain crucible. One hundred grams of mercury, previously heated to above 30°C below the probable melting point of the resin, was poured in over the cooled sample, and a thermometer immersed. After heating the crucible slowly, the temperature at which the first resin appeared at the surface of the mercury was reported as the melting point. The results are shown in Table 6 below:

Table 6

| Method of Collection | Melting Point |
|---|---|
| "Scrape" | 68°C |
| "Chip" | 66°C |
| "Bore" | 65°C |

## C. Physical Properties of the Purified Oil

### 1. Specific Gravity

Specific gravity is an important criterion of the quality and purity of an essential oil. Of all the physiochemical properties, the specific gravity has been reported most frequently in the literature. Values for volatile oils usually vary between the limits of 0.696 and 1.188 at $15^0$C. In general it is less than 1.000.

Langenau (1948) contends that for determination of this physical property, accuracy to at least the third decimal place is necessary. Therefore, hydrometers are of little value, and should not be used. Pycnometers offer the most convenient and rapid method for determining specific gravities, but are not useful where the amount of oil is limited. Sprengel or Ostwald tubes give more accurate results, and can be used with small quantities of oil. However, a determination can not be run as rapidly or as conveniently.

For the specific gravity determinations in this study, a small, modified Sprengel tube having a capacity of 0.2967 cubic centimeters was made. The tube was weighed empty, filled with mercury, and filled with the oil being tested. All weighings were carried out on an analytical balance at

$25^0$ C, and were calculated to the fourth decimal place. Between sample determinations, the weighing tube was rinsed successively with alcohol, acetone, and ether, and allowed to dry completely before proceeding with any further determinations.

The specific gravity of the oils from each of the three collections is shown in Table 7.

Table 7

| Method of Collection | Specific Gravity |
|---|---|
| "Scrape" | 0.8520 |
| "Chip" | 0.8683 |
| "Bore" | 0.8543 |

2. Solubility In Alcohol

Since most volatile oils are only slightly soluble in water and are miscible with absolute alcohol, the number of volumes of dilute alcohol required for the complete solubility of one volume of oil forms a convenient and rapid aid in the evaluation of the quality of an oil. In general, oils rich in oxygenated constituents (except those belonging to the

sesquiterpene series) are more readily soluble in dilute alcohol than oils rich in terpenes (Langenau, 1948).

The solubility of the oils from pinyon oleoresin in ninety-five per cent alcohol was determined at 25° C by the method of Langenau (1948).

The following observations were the same for the three oils: cloudy to turbid upon addition of from one to six volumes of alcohol, becoming clear only after the addition of ten volumes of alcohol. After twenty-four hours all samples became slightly hazy.

3. Refractive Index

The refractive index of the oils was determined using an Abbé refractometer. This type of instrument, with a range of 1.3 to 1.7 is recommended (Langenau 1948) for the routine analysis of essential oils, the accuracy of this instrument being adequate for all practical work.

The readings were made directly at 25°C using the procedure described in U.S.P. XVI. Results are shown in Table 8.

Table 8

| Method of Collection | Refractive Index |
|---|---|
| "Scrape" | 1.4740 |
| "Chip" | 1.4713 |
| "Bore" | 1.4753 |

4. Optical Rotation

Most volatile oils, when placed in a beam of polarized light, possess the property of rotating the plane of polarization to the right (dextrorotatory) or to the left (laevorotatory). The extent of the optical activity of an oil is determined by a polarimeter, and is measured in degrees of rotation. Both the angle of rotation and its direction are important as criteria of purity and identification.

The angle of rotation depends upon the nature of the liquid, the length of the column through which the light passes, the wave length of the light used, and the temperature.

The optical rotation for each purified oil sample in this study was determined in a 100 mm semi-micro tube using a Rudolph polarimeter of the half-shadow type. A sodium vapor lamp was used as a light source.

Even though a standard temperature of $20^0$C is usually adopted for volatile oils in the literature, the U. S. P. and N. F. specify $25^0$C as the official temperature for all optical rotations. For this reason, all determinations were carried out at $25^0$C according to the method described in U. S. P. XVI.

By using the semi-micro tube, readings could be taken directly in preference to making dilutions in order to fill the regular polarimeter tubes, and then being required to calculate the specific rotations using a correction factor for concentration.

The rotatory power and direction of rotation of the oils is shown in Table 9.

Table 9

| Method of Collection | Optical Rotation |
|---|---|
| "Scrape" | $+ 28^0 36'$ |
| "Chip" | $+ 28^0 41'$ |
| "Bore" | $+ 27^0 20'$ |

5. Organoleptic Properties

Organoleptic refers to evaluation by means of the organs of sense. This includes the macroscopic appearance

of the drug, its odor and taste, the sound or "snap" of its fracture, and the "feel" of the drug to the touch. These characteristics can not be measured quantitatively, nor is there any standardized nomenclature by which these subjective impressions can be described. Nevertheless, these properties are of considerable importance in the manufacture of pharmaceuticals because the impressions impinged upon the senses may determine whether a product is acceptable or not. In the case of the pinyon oils, their odor was considered both distinctive and pleasant. They should, therefore, be acceptable as perfuming agents in preparations such as soaps, bath oils, and deodorizers. The value of these oils as flavors was considered doubtful because of their acrid, terebinthic taste.

An attempt has been made to characterize these properties in Table 10. All of the oils tested were indistinguishable, and therefore were considered together.

6. Non-Volatile Residue

To test for the presence of non-volatile material, a drop of oil was placed on a piece of white paper and allowed to evaporate at room temperature. A positive test is indicated

Table 10

| Organoleptic Properties | |
|---|---|
| Color | Clear - Colorless to pale straw color |
| Odor | Pleasantly pinaceous with a slight but characteristic pinyon odor |
| Taste | Acrid, Turpentine-like |

by a residual stain on the paper after the odor of the oil can no longer be detected. No non-volatile residue could be detected in the analysis of the pinyon oils under consideration.

# VII. PHARMACEUTICAL PREPARATIONS

Reports attesting to the curative qualities of pinyon oleoresin have been cited previously. These reports also indicate that there has been little, if any, effort made to enhance the acceptability of pinyon oleoresin for use as medication. Indeed, in most cases the natural exudate was consumed directly from the tree, in spite of organoleptic objections.

Research was undertaken to develop acceptable pharmaceutical formulations and applications of refined pinyon oleoresin. The stimulus for this research came from two principal sources: first, from the number of people currently using the crude material for the treatment of both internal and external ailments; and second, from the possible economic potential of a line of marketable medicaments of this type.

## A. Preparations For Local Application

Preparations for local or external application are liquid or semi-solid in nature, and of such consistancy that they may be readily applied to the skin by inunction. They serve as vehicles for the topical application of medicinal substances. They may be oleaginous mixtures of fatty

substances or emulsions of fatty or wax-like materials with comparatively high proportions of water. These emulsions may be either of the water-in-oil (W/O) or oil-in-water (O/W) type, depending primarily upon the selection of the emulsifying agent.

1. Water-removable Oleoresin Ointment

The base employed was an oil-in-water emulsion, as is the case with most "water-washable" ointments. The preparation may be readily washed from the skin or clothing with water, an attribute that makes them more acceptable for cosmetic reasons. Other advantages of water-removable bases are that they may be diluted with water, and that they favor the serous discharges in dermatological conditions.

Hydrophilic ointment was the base employed in this case. One hundred grams was prepared according to the procedure outlined in U.S.P. XVI:

| | |
|---|---|
| Methylparaben | 0.25 Gm. |
| Propylparaben | 0.15 Gm. |
| Sodium Lauryl Sulfate | 10.00 Gm. |
| Propylene Glycol | 120.00 Gm. |
| Stearyl Alcohol | 250.00 Gm. |
| White Petrolatum | 250.00 Gm. |
| Purified Water | 370.00 Gm. |

Melt the stearyl alcohol and the white petrolatum on a steam bath, and warm to about 75°C. Add the other

ingredients, previously dissolved in the water and warmed to 75°C, stir the mixture until it congeals.

Ten grams of oleoresin was incorporated into this base by fusion. The resulting mixture was a smooth, homogeneous mass which could be termed a "therapeutic cream" (Zopf, 1961). When the preparation was applied locally with inunction, it caused noticeable erythema in the test area.

2. Oleaginous Oleoresin Ointment

This preparation is one in which the pinyon oleoresin was incorporated into a hydrocarbon base. This type of base serves to keep medicaments in prolonged contact with the skin and acts as an occlusive dressing. Hydrocarbon bases are used chiefly for their emollient effects, and are difficult to wash off. They do not change noticeably on aging, nor do they "dry out".

White Ointment was chosen as the oleaginous base. One hundred grams of the base was prepared according to directions outlined in U. S. P. XVI:

| | |
|---|---|
| White Wax . . . . . | 50 Gm. |
| White Petrolatum. . . . . | 950 Gm. |
| To make . . . . . | 1000 Gm. |

> Melt the white wax in a suitable dish on a water bath, add the white petrolatum, warm until liquefied, then discontinue the heating, and stir the mixture until it begins to congeal.

Fifteen grams of the oleoresin was incorporated into one hundred grams of the oleaginous base by fusion. The mixture formed a smooth, homogeneous mass. As with the water-washable ointment, this preparation caused erythema as evidenced by redness of tissue in the test area which lasted for from fifteen to thirty minutes after applying the ointment by inunction.

3. Cerates

These are unctuous substances which owe their name to the presence of wax (cera). They are of such consistency that they may be easily spread at ordinary temperatures, upon muslin or similar material with a spatula, and yet not so soft as to liquefy and run when applied to the skin. Rosin, paraffin, and spermaceti are often used to raise the melting point of oils and fats in the formulation of cerates.

To compare the stiffening properties of pinyon resin with that of rosin, two samples of Rosin Cerate (N.F. VIII) were prepared. Both preparations were made according to the specifications of the National Formulary, except in one

case the pinyon resin was used in place of rosin. The procedure as specified by the N.F. VIII is as follows:

| | |
|---|---|
| Rosin . . . . . | 350 Gm. |
| Yellow Wax . . . . . | 150 Gm. |
| Lard . . . . . | 500 Gm. |
| To make . . . . . | 1000 Gm. |

Melt the rosin together with the yellow wax and lard. Stir and strain until congealed.

When the two cerates were compared, it was noted that the pinyon resin formulation became softer than the standard formula when both were applied to the skin. Also, the pinyon preparation was lighter in color and had the characteristic pinyon odor. The pinyon resin cerate was considered to have no particular advantage over the standard rosin formula, except in cases where a "softer" preparation might be desired.

4. Dental Cavity Liners

On the basis of reports in the literature, it was suggested that pinyon resin varnish should be investigated regarding its value in a protective coating. Colton (1948) reported using a varnish made from pinyon resin successfully on one of her paintings and as an outdoor and indoor varnish on wood. She painted pieces of pineboard with the varnish

and exposed them to the northern Arizona wind, rain, snow, frost, and sunshine with excellent results. M. W. Westgate (1954) reported pinyon resin as having a potential market value as an ingredient in both spirit-type and oleoresin type varnishes because of its superior resistance to both hot and cold water.

The investigation of pinyon resin as a component of dental varnishes was considered important in light of these reports. In addition, Swartz (1961) credits the use of a cavity varnish with improving the sealing ability of cavity liners. She also stated that a copal resin varnish established a relatively good initial seal which did not change materially during the period of the study.

Cavity liners or varnishes are used to seal the dental tubuli in deep-seated cavities so as to protect the pulp from acidic dental cements. Most cavity varnishes are solutions of copal resin or rosin in a volatile solvent such as chloroform.

A pinyon cavity liner and a traditional rosin cavity liner were prepared according to the procedure set down in Accepted Dental Remedies (1960):

Rosin, fragments . . . . . 7 Gm.
Chloroform . . . . . 100 ml.

Make a solution.

The standard and the pinyon preparations were submitted for comparison in the general practice of a local dentist (Clark, 1961). After two months examination of cavity preparations lined with these varnishes, it was concluded that the pinyon liner was at least equally as effective as the standard rosin liner. Indeed, it was suggested that on the basis of this preliminary study, the pinyon resin liner be submitted to the Council on Dental Therapeutics of the American Dental Society for a complete chemical and dental evaluation.

## B. Preparations For Internal Use

### 1. Emulsions of Oleoresin

Drugs containing oils are best prepared for oral administration by first emulsifying them in a flavored, sweetened, aqueous medium. Only oil-in-water emulsions are suitable for oral use, since they are water-miscible and thus their "oiliness" is masked. The U.S.P. XVI describes emulsions as follows:

> An emulsion is a dispersed system in which one liquid, termed the "dispersed" or "internal" phase, is distributed in small globules throughout the body of the second liquid, termed the "dispersion

> medium" or "external" phase. Where oil is the dispersed phase and an aqueous solution is the dispersion medium, the emulsion is said to be an oil-in-water emulsion. Such an emulsion is miscible with water. Where water or an oleaginous material is the dispersion medium, the emulsion is described as a water-in-oil emulsion, and is miscible with oil.

Because of the terebinthic, acrid taste of the oleoresin, the oil-in-water type of emulsion was formulated in an effort to create an acceptable preparation. The first attempt at emulsifying the oleoresin was by first dissolving it in equal parts of mineral oil with acacia as the emulsifying agent, and cherry syrup as the aqueous vehicle. This preparation, although pleasant tasting, could not be maintained in the emulsified state.

The second attempt to form a liquid preparation which could be taken orally, was to dilute the hydrocarbon base ointment with glycerin to form a thick liquid. Lemon oil emulsion was used in an effort to mask the disagreeable taste of the oleoresin. The mixture of lemon oil emulsion and glycerin liquid was passed through a hand homogenizer to yield an emulsion with satisfactory physical properties. However, the taste was considered to be unacceptable.

The following formulation was developed, and was considered to be acceptable. The ingredients and procedure used are given below.

(a) Liquified Pinyon

| | |
|---|---|
| Pinyon oleoresin, refined | 80 ml. |
| Peanut oil | 20 ml. |

The oleoresin and oil were heated on a water bath to a temperature not exceeding $70^0$C. The mixture was stirred until the fusion was complete and finally until the solution had cooled.

(b) Primary emulsion

| | |
|---|---|
| Oleoresin-oil mixture | 40.0 ml. |
| Lemon oil | 5.0 ml. |
| Tween 80 | 16.0 ml. |
| Span 80 | 8.0 ml. |
| Saccharin, U.S.P. XVI | 0.5 ml. |
| Purified water to make | 100.0 ml. |

The emulsifying agents, saccharin, lemon oil, and the pinyon oil mixture, were incorporated together in a wedgewood morter. After a homogeneous mixture had formed, the water was added slowly until an emulsion developed. The emulsion was then passed through a hand homogenizer.

(c) Final product

| | |
|---|---|
| Primary emulsion | 50.0 ml. |
| Glycerin | 30.0 ml. |
| Cocoa | 20.0 Gm. |
| Saccharin, sodium | 1.5 Gm. |
| Glycerin to make | 100.0 ml. |

A mixture was formed with the glycerin, cocoa, and soluble saccharin. The resultant combination was then added slowly and with vigorous stirring to the primary emulsion. This combination formed a sweet chocolate flavored syrup which was considered acceptable for oral administration.

2. Capsules of Oleoresin

Capsules are described by the U.S.P. XVI as follows:

> Capsules are solid dosage forms in which the drug is enclosed in either a hard or a soft, soluble container or "shell" of a suitable form of gelatin. Capsules sizes range from No. 5, the smallest, to No. 000, which is the largest, except for veterinary sizes. Factory-filled hard capsules are often of distinctive color and shape or are otherwise marked to identify them with the maker.
>
> In extemporaneous prescription practice, the use of capsules permits a latitude of choice in prescribing either a single drug or a combination of drugs at the precise dosage level considered best for the individual patient by his physician. This flexibility gives capsules an advantage over tablets as a dosage form.

In addition to the advantages cited by the U. S. P., capsules offer a convenient means of administering concentrated drugs orally regardless of their odor or taste. Capsules are a desirable dose form in that they may be carried on the person in purse or pocket. However, for small children, or adults who can not swallow solid medication, a liquid formulation is more suitable.

The semi-solid nature of pinyon oleoresin precluded its incorporation by the usual "dry pack" techniques. In fact, the only method of utilizing the oleoresin in its natural state (except for refinement) was to soften it with gentle heat and then roll the mass into a "pill-pipe". To prevent the cylinder from sticking to the work surface, and to lessen friction, a starch dusting powder was used. The diameter of the "pipe" was adjusted so that it could be inserted into a hard gelatin capsule of appropriate size. Thereupon the cylinder was cut into desired lengths, the pieces placed into the empty capsules, and finally the caps replaced.

For a drug to be acceptable in any dose form, it must have widespread distribution and be sold at a reasonable price. In the case of pinyon oleoresin, it was obvious that hand made capsules could not be supplied in quantity and would

have to be priced prohibitively high as well. Therefore, a method was needed which could be extended to commercial production.

Encapsulation machines currently available to the pharmaceutical manufacturers require that the drug be either in powder or liquid form (Jarowski, 1961). To ascertain the adaptability of pinyon oleoresin for automatic manipulation, the oleoresin was liquified with peanut oil in the same manner as it was for the emulsion. A medicine dropper was used to meter the correct amount into the empty capsules. The caps of the capsules were then sealed in place by moistening the inner surface with a cotton tip applicator, the cotton being saturated with diluted alcohol. Empty capsules as small as No. 5 were filled without difficulty. The only problem in effecting a good cap seal was in those cases where the oily mixture had come in contact with the outside of the capsule, thus causing leakage. Except in these cases, the capsules appeared to be in good condition after several months time.

3. Water, aromatic

Aromatic waters, also known as medicated water, may be defined as clear, saturated aqueous solutions of volatile

oils or other aromatic or volatile substances. Their odor and taste are similar to those of the drugs or volatile substances from which they were prepared (U. S. P. XVI).

Waters may be prepared by one of three official processes: (1) simple solution in cold water, (2) solution with the aid of a distributing agent, and (3) by distillation. The distillation process is the most ancient and often the most satisfactory method of manufacturing this class of preparations (Ehrenstein, 1961).

The U. S. P. XVI directions for the distillation method are as follows:

> Place the odoriferous portion of the plant or drug from which the aromatic water is to be prepared in a suitable still with sufficient purified water, and distil most of the water, carefully avoiding the development of empyreumatic odors through the charring or scorching of the substances. Separate the excess oil from the distillate, and preserve or use the clear water portion, filtering if necessary.

The oleoresin water had a characteristic pinyon odor which was not unpleasant. However, the taste was acrid and terebinthic. Consequently, the water would probably be of little value as a pharmaceutical adjunct.

# VIII. DISCUSSION

## A. General

Species of pinyon are found only in the western and south-western part of North America, and to a limited extent in Central America. The most abundant species, as well as those most frequently reported in the literature, are Pinus edulis and Pinus monophylla. Pinus edulis ranges from western Texas through New Mexico, Arizona, and into east-central Utah; and Pinus monophylla can be found from eastern California and northern Idaho south and east to central Utah. An ecotone extending from central to south-western Utah, has been described by Cole (1956). Individual trees exhibiting the morphological characteristics of both species were observed.

Histological sections were examined to determine the concentration, size, and location of the resin ducts. On this basis it was seen that large resin ducts are found in the cortical region and that they are several times as large as those found in the xylem. In view of these findings it was concluded that scarification need not be deeper than the cambium zone. To give further support to this premise, a branch

was cut transversely and the zone of greatest oleoresin production was also noted to be peripheral to the cambium zone.

A comparative evaluation of the histological sections was done to determine if there were any outstanding morphological characteristics by which the intermediate form could be differentiated from Pinus edulis and Pinus monophylla. The leaf sections showed identical characters in morphologic equals. The bark of the three types was nearly identical. However, the bark of Pinus monophylla contained larger resin ducts than the others. The xylem of all three forms appeared to be identical.

The intermediate form became of particular interest as a result of several reports regarding the medicinal use of the oleoresin from this tree. It was reported as being effective in the treatment of numerous gastro-intestinal disturbances, and also when applied externally as a "drawing" agent, an antiseptic, and as a healing salve.

The oleoresin which had been used is that which accumulated as a natural exudate at the site of some injury on the tree. This exudate builds up over several years, with coincidental oxidation by the air and sun, and polution by

all manner of impurities. The investigation and development of different methods of collection and refinement, and the utilization of pinyon oleoresin in acceptable pharmaceutical preparations, formed the basis of this study.

## B. Collection, Refinement, and Analysis

Three methods of collection were described: scraping the naturally occurring exudate from the tree ("scrape" oleoresin), chipping the trees to expose the resin ducts and then directing the flow with metal gutters into a can ("chip" oleoresin), and boring holes into the wood of the tree, then fitting a plastic vial of appropriate size into the holes for collection of the exudate ("bore" oleoresin).

Samples, collected by the methods described, varied considerably in the amounts of impurities or trash which they contained. The "bore" collection was a clear, colorless liquid which required no refinement. The "chip" collection was a white, opalescent, viscous liquid with varying amounts of contamination; thus necessitating some degree of refinement. The "scrape" oleoresin was mixed with considerable quantities of debris, and therefore required more extensive refining.

The difference in yields from the "bore" and the "chip" methods for the three individual periods was not consistent. However, the time of year in which the collections were made seems to be a relevant factor. These yields were shown to be largest in the fall, and less in winter or early summer. The "bore" collection was greater than the "chip" in early summer, while the "chip" collection was greater during the fall period.

This seasonal variation might be explained on the basis of a difference in metabolic rates, availability of photosynthate, or by the effect of available water on turgor pressure.

Because of the possible loss or alteration of therapeutically active constituents, the refining methods were restricted to the use of gentle heat and straining. In order to prevent the oleoresin from congealing on the screening device, a sieve was shaped to conform to the dimensions of the heating vessel. In this way, the wire mesh was heated by the oleoresin which it contained, and also by the vessel which surrounded it. All but the smallest particles were removed by raising the sieve up through the liquified material.

The refined oleoresin was distilled with water to separate the volatile oil and resin fractions. The first

volatile product evolved at 163°C. After six to eight hours of hydrodistillation, extraction was complete. The oil and resin from the three different collections were compared as to yield and certain physical and organoleptic properties. These are summarized in Tables 11 and 12.

## C. Preparations

Pharmaceutical preparations were made for both external and internal administration. Ointments of both hydrocarbon and oleaginous type were formulated using pinyon oleoresin as the medicament. Rosin cerate was made according to the procedure outlined in the N.F. VIII. A cerate was also made by this method using pinyon resin in place of rosin. A dental cavity liner was made using pinyon resin in place of the usual rosin. This cavity varnish was formulated according to Accepted Dental Remedies.

For internal use, capsules containing the refined oleoresin were made by forming a "pill-pipe" and then cutting and inserting the portions into empty gelatin capsules. Capsules were also made by liquifying the oleoresin with peanut oil and then instilling the mixture into empty gelatin capsules with a medicine dropper. A liquid preparation for internal

Table 11

Volatile Oils

| Properties | "Bore" Collection | "Chip" Collection | "Scrape" Collection |
|---|---|---|---|
| Percent yield | 18.85 | 14.80 | 11.53 |
| Specific Gravity | 0.8543 | 0.8683 | 0.8520 |
| Optical Rotation | + 27°20' | + 28°41' | + 28°36' |
| Refractive Index | 1.4753 | 1.4713 | 1.4740 |
| Non-volatile Residue | none | | |
| Solubility in Alcohol | 6 vol. - Cloudy to turbid<br>10 Vol. - Clear | | |
| Color | Clear-colorless to pale straw color | | |
| Odor | Pleasantly pinaceous with a slight but characteristic pinyon odor | | |
| Taste | Acrid - terebinthic | | |

Table 12

Resins

| Properties | "Bore Collection | "Chip" Collection | "Scrape" Collection |
|---|---|---|---|
| Acid Number | 163.6 | 156.8 | 132.4 |
| Saponification No. | 173.9 | 168.0 | 170.5 |
| Drop Softening Point | 55 - 57°C | 55 - 58°C | 57 - 58°C |
| Melting Point | 65°C | 66°C | 68°C |

use was made by incorporating the oleoresin-peanut oil mixture to form a primary emulsion. A syrup was then formed by adding glycerine, saccharin sodium, and cocoa.

Both types of ointments were considered acceptable. However, there were small foreign particles present which had not been removed by straining. These impurities were particularly noticeable in the washable preparation. When applied to the skin, the ointments left an oily film, with that from the oleaginous preparation being the most conspicuous. The odor of both ointments was not objectionable.

Although the pinyon cerate was an acceptable preparation, there is little demand for this type of pharmaceutical in current medical practice.

The capsules formed the most practical form of oral preparation. Both "pill-pipe" and "liquid" filled capsules remained stable for several months. The question arises as to how large a capsule would be required to administer an adequate dose of the oleoresin. On the basis of the reports by users, it would probably require several No. 00 capsules per day. This is an objectionable size.

The liquid, oral dose form was the only formula tried which would tolerably cover the terebinthic after-taste.

However, this preparation may seem excessively sweet to some.

## D. Proposed Evaluations

The advisability of introducing a new medicinal product onto the ethical pharmaceutical market hinges on several factors: (1) a need for the curative power claimed for the drug, (2) demonstrated superiority of the drug over existing agents used to treat the same disease entities, (3) pharmacological evaluation to determine therapeutic index, absorption, fate, and excretion of the drug, and (4) clinical evaluation to ascertain the true effectiveness of the drug in human patients under controlled conditions.

On the basis of the criteria listed above, if pinyon oleoresin were shown to contain some therapeutically active constituent, the next step should be to ascertain the best conditions and plant source for the oleoresin. Before entering into the production of pharmaceuticals it would also be advisable to determine if the active constituents were in the oil or the resin fraction. On the basis of these findings, the formulation of elegant preparations and production engineering could proceed.

# IX. SUMMARY AND CONCLUSIONS

Pinyon oleoresin has been reported by several laymen as having curative effects when used against various ill-defined gastro-intestinal disorders. It has also been reported that the oleoresin is effective as a "drawing" salve and in promoting healing when applied locally. Because of the gummy nature of the natural exudate, it was suggested that if acceptable pharmaceutical preparations were made, a potential market might exist for medicaments containing pinyon oleoresin.

The oleoresin for this research was collected from an intermediate variety of pinyon exhibiting morphological characteristics of both Pinus edulis and Pinus monophylla. Three methods of collection were investigated: (1) scraping the naturally occurring exudate from the tree, (2) scarifying or chipping the tree and then directing the flow of material with a metal gutter into a cup, and (3) by boring into the wood and collecting the oleoresin with a plastic vial fitted into the hole. Comparisons of the yields indicate that the cup and gutter system produced considerably more oleoresin than the other two systems, and also that the oleoresin could be collected in a relatively pure state.

The most efficient method of removing foreign material was to place the crude oleoresin into a straining device of approximately the same size and shape as the heating vessel. Upon application of enough heat to liquify the raw material, the wire mesh straining device could be raised up through the heated oleoresin, thereby removing any undesirable particles.

Analysis of the volatile oil and resin fractions showed the "bore"collection to have the highest percent of volatile oil, and the "scrape" to have the least. All three oil fractions were clear, colorless to pale straw color, having a pleasant, pinaceous pinyon odor and an acrid, terebinthic taste.

The resins from the "bore" and "chip" samples were lighter in color than the "scrape" resin. The drop-softening points of the three samples ranged from $55^0$ to $58^0$C, and the melting points from $65^0$ to $68^0$C.

Acceptable pharmaceutical preparations for both internal and external use were made utilizing the oleoresin in the formulations. Hydrocarbon and oleaginous base ointments were manufactured. A cerate was made using the resin in place of rosin in an official formula. A dental cavity liner was made which seemed to have superior sealing qualities

when compared with a standard rosin formula. Capsules were made by manipulating the oleoresin so it could be inserted into empty gelatin capsules, and by liquifying the oleoresin with peanut oil, thus making it possible to instill the mixture into empty gelatin capsules with a medicine dropper. A pleasant tasting chocolate preparation was made by forming a primary emulsion with the oleoresin-peanut oil mixture and then adding the vehicle and flavoring agents to form a syrup.

It is concluded that pinyon oleoresin can be collected and refined in quantity; also, that acceptable pharmaceutical preparations can be formulated for both internal and external administration of the oleoresin. However, before attempting any large-scale production and marketing, it would seem advisable to submit the oleoresin and its oil and resin fractions to carefully controlled studies regarding not only their therapeutic efficacy, but also the establishment of a pharmacological basis for the activity which has been reported. In addition, the toxicity of the preparations or their constituents (such as emulsifying agents) should be determined by chronic studies.

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